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14. ABSTRACT

Over 26% of our military personnel injured in combat have severe fractures of their arms and legs. Civilians sustain similar injuries in motor vehicle collisions, falls, and after gun shot injuries. One of the goals of treatment is to have the body replace or restore lost bone. While bone usually heals without complication, about 10% of patients go on to have no healing or really slow healing. Over the past 20 years, knowledge of bone healing has increased with discovery and use of proteins and growth factors. However, there has been no description of the role of **Notch** signaling in fracture healing. **Notch** is involved in turning on cells that make bone and provide blood supply. We believe that by increasing the amount of **Notch** at a fracture site, injured bones in our military and civilian patients will heal better and faster. We will look at Notch in human and mouse fractures and enhance fracture healing by putting Notch on a special sponge to deliver it into an area of injury. We believe that developing tissue engineering applications to increase Notch signaling will promote healing, in combination with what surgeons are already doing. Once we show how Notch works in humans and enhance fracture healing in mice by increasing Notch delivery, a large animal model (e.g., sheep) could be developed and tested in another 2 years. Within five years, we will have a product to help soldiers on the battle field with fractures and bone defects. The risks of this therapy are low since Notch is normally produced in the body.

15. SUBJECT TERMS
None provided.

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Table of Contents

Page

Introduction

Notch signaling has been shown to regulate the maintenance and differentiation of stem cells and influences both wound healing and angiogenesis. Fracture healing has parallels to skin wound healing, and is influenced by both rate and extent of angiogenesis and by the mesenchymal progenitors that proliferate and differentiate to become bone forming osteoblasts. Recent published reports show that Notch activation promotes bone formation in young mice by increasing the number of osteoblast progenitors. Our preliminary results show that activation of Notch signaling by plating cells onto the Jagged-1 ligand increases progenitor cell number. Additionally, our preliminary results show that there are increases in Notch receptors and Jagged-1 ligand expressed in fracture tissue and concomitant increases in Notch target gene expression.

To address this hypothesis, we proposed three specific aims. In the first aim, we are examining Notch signaling in both human and murine fracture healing. In the second specific aim, the physiological significance of Notch signaling will be examined in murine fracture healing through blocking Notch signaling locally by driving the expression of dominant negative mastermind like protein (dnMAML) or through stimulation of Notch signaling by driving the expression of the Notch intracellular domain (NICD). Finally, in the third specific aim, we will develop a tissue engineering strategy using a unique osteogenic, biodegradable biomaterial, A6, to deliver Jagged-1 ligand to promote bone regeneration.

Body

The first aim of the grant was to characterize Notch signaling in human long bone fractures. To test the hypothesis that Notch signaling is physiologically relevant in human fractures and that alterations in Notch signaling are associated with delayed and non-union, we have collected specimens from relevant fractures at various time-points including non-union, delayed unions, and normal healing. IRB approval for the prospective component of the study has been obtained and active enrollment is ongoing. Thus far, over 20 prospective specimens have been collected and stored for future analysis. Patient enrollment is at a level that is consistent with our study goals and fracture characteristics that are relevant to our inclusion and exclusion criteria. Patient enrollment will continue throughout the period of the grant.

The next steps will be to evaluate expression of Notch receptor, ligand, and target genes in human tibial fractures from this patient population and correlate those to their clinical outcome. In an effort to minimize redundancy, prospective analysis of samples will be performed when we have reached a threshold number of specimens with clinically relevant endpoints such as fracture healing or non-union.

In addition, over the course of the last year, we have obtained waste material from fracture surgery which we will review in a retrospective fashion for Notch expression.

Moving forward, the tissue we have collected will be evaluated by crushing it using a mortar and pestle and then further macerating it using a Tissue Tearor (Biospec Products). RNA will be isolated using the Qiagen RNeasy Mini kit (Qiagen, Valencia, CA). RNA yield will be determined spectrophotometrically and integrity confirmed by gel electrophoresis. cDNA will be synthesized from 0.5 µg of mRNA from each sample. The expression levels of the following genes will be determined using a 7500 Fast real time thermocycler (Applied Biosystems): Notch(1-4), Jag-1,2, Dll-1,3,4, Hey1,2,L, and Hes1,2. RNA fold expression levels will be calculated using the double delta-CT method, and proper amplicon formulation confirmed by melt curve analysis and gel analysis. Tissues for immunohistochemistry will be decalcified, embedded in paraffin and sectioned at 7 µm onto charged slides. Cleaved (activated) NICD will be examined. Following treatment with primary antibody, sections will be treated with Alexafluor 594 labeled secondary antibodies, mounted with Vectashield containing DAPI (Vector Laboratories), and visualized with a fluorescent microscope. Expression of cleaved Notch can then be further classified based on cell phenotype (macrophage, endothelial cell, osteoblast, chondrocyte) using dual antibody labeling with antibodies for specific cell types. Additionally, we will use IHC to evaluate the most prevalently expressed ligand(s) and Notch receptors as determined by gene expression.

Key Research Accomplishments

IRB Approval with HIPAA approved consent from the University of Pennsylvania for prospective enrollment.

Completion of VA Mandated Research Training Program.

Bimonthly research meetings with research team including Kurt Hankenson, PhD and Jason Burdick, PhD.

Pending VA IRB Approval for prospective study at VA Hospital.

Aggregation of samples of waste material from fracture surgery without clinical correlation.

Assignment of a research coordinator to review daily inpatient and surgical lists for potential subjects.

Enrollment of over 20 subjects with acute injuries.

Ongoing collection process including nights and weekends.

Reportable Outcomes

Reportable outcomes for Aim I – notch levels, notch activity, presence of ligand – pending until analysis of sufficient number of specimens

Conclusion

Pending as we are in Year 1 of 3 year grant, but specimen collection is underway at a brisk level.

References

None

Appendices

None